American Journal of Medical Genetics 63:509–517 (1996)

Tel Aviv-Heidelberg Three-Generation Offspring Study: Genetic Determinants of Plasma Fibrinogen Level

G. Livshits, G. Schettler, E. Graff, M. Blettner, J. Wahrendorf, and D. Brunner

Department of Anatomy and Anthropology (G.L.), Institute of Physiological Hygiene, Wolfson Medical Center (E.G., D.B.), and Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; Division of Epidemiology, German Cancer Research Center (M.B., J.W.), and Geomedical Research Unit, Heidelberg Academy for Humanities and Sciences (G.S.), Heidelberg, Germany

Elevated plasma fibrinogen concentrations (fibrinogen) are an important independent risk factor of atherosclerotic disease. Using the kinetic method, we measured fibrinogen in 808 individuals, of which 757 were members of 204 pedigrees. Correlation analysis and two-way analysis of variance (ANOVA) showed a significant association of fibrinogen with age, body mass index (BMI), sex, smoking habits, sport activity, and other lifestyle factors. However, multivariate regression analysis of fibrinogen established an independent significant contribution of only the first three factors. Fibrinogen levels adjusted respectively were subjected to complex segregation analysis. Our aim was to identify the contribution of major gene effects and residual (within the genotype) family correlations on fibrinogen variation. Results of this study suggest codominant alleles at a major locus accounting for 39% of variation. There was also evidence of a significant residual parent/offspring correlation.

KEY WORDS: major gene, transmissibility, segregation analysis

INTRODUCTION

Human fibrinogen is a long glycoprotein which is synthesized predominantly in the liver. Fibrinogen is an acute-phase protein and a clotting factor. In addition it has numerous other functions, e.g., it is an essential cofactor for platelet aggregation, and it determines the rheological behavior of blood [Dang and Bell, 1989; Ernst, 1990]. It has been shown that plasma fibrinogen concentration is an independent and significant predictor of incident clinical atherosclerotic diseases, including coronary disease, peripheral vascular disease, and stroke [Wilhelmsen et al., 1984; Kannel et al., 1987; Fowkes et al., 1992; Ernst, 1993].

Several studies have reported that fibrinogen levels depend on age, sex, smoking habits, obesity, socioeconomic status, and other environmental factors [e.g., Ernst, 1993; Folsom et al., 1993]. Folsom et al. [1993] also demonstrated significant racial differences in fibrinogen levels, which could be indicative of the involvement of genetic factors. Indeed, a genetic determination of fibrinogen levels seems to exist [Hamsten et al., 1987; Humphries et al., 1987; Mihai and Jansco, 1991].

For example, the study by Mihai and Jancso [1991] suggests a significant association between parents' and children's fibrinogen levels. Hamsten et al. [1987], using path analysis in 170 nuclear families, suggested that 51% of the total variance in plasma fibrinogen levels is attributable to additive genetic factors. Humphries et al. [1987] performed analysis of fibrinogen concentrations using fibrinogen alpha and beta loci on 91 healthy English subjects and concluded that genetic variation at these loci accounted for 15% of total variance. In contrast, Berg and Kierulf [1989] detected no association in 118 Norwegian subjects between levels of plasma fibrinogen and the fibrinogen genotypes expected grounding on DNA polymorphism. Furthermore, Thomas et al. [1991], who analyzed the *Hae*III polymorphism of the beta fibrinogen gene in 292 healthy subjects, were able to explain about just 3% of the variance in fibrinogen levels. Finally, a more recent study by Connor et al. [1992] examined four DNA polymorphisms at the alpha, beta (two loci), and gamma fibrinogen loci in 247 individuals. Plasma fibrinogen was determined by clotting and nephelometric assays. However, plasma fibrinogen levels by either assay method did not show any statistically significant associations with the examined marker loci, whether singly or when analyzed as haplotypes.

Thus, the above-mentioned studies show a great discrepancy in results of genetic analysis of fibrinogen-

Received for publication May 8, 1995; revision received February 6, 1996.

Address reprint requests to Gregory Livshits, Ph.D., Department of Anatomy and Anthropology, Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv 69978, Tel Aviv, Israel.

level variation. To clarify this issue, we undertook complex segregation analysis of a large Israeli sample of healthy individuals, belonging to about 200 nuclear and extended pedigrees.

MATERIALS AND METHODS

Sample and Variables

The data collection in the present study was carried out during 1992–1994 on the survivors and relatives of the cohort recruited for the Donolo-Tel-Aviv prospective coronary artery disease study in 1964 [Brunner et al., 1987]. All individuals were clinically healthy at entrance examination. The present sample includes 1,490 subjects with 808 individuals of both sexes, on whom measurement of plasma fibrinogen level was performed. The age of individuals varied between 6–94 years, with an average age of 46.8 years, and individuals included survivors of the cohort recruited in 1964 (first generation), their married children and in-laws (second generation), and grandchildren (third generation). The size of pedigrees varied from 2 (parent-child, siblings) to 14, representing quite complex pedigrees. A few examples of the latter are shown in Figure 1. Fifty-one individuals in the sample represented a single individual family. They were used for analysis of the relationship between fibrinogen level and age, sex, and several of the life-style variables listed below. However, for purposes of genetic analysis, they were deleted. Thus, the final sample size was 757 individuals belonging to 204 pedigrees.

Plasma fibrinogen level determination was performed by the kinetic method with Boehringer-Mannheim analytical kits (Germany), on a Boehringer-Hitachi 704 analyzer (Germany) [Hemker et al., 1979].

In addition to fibrinogen measurement, body weight and height, as well as basic socio-demographic data, i.e., age, sex, education, occupation, physical exertion at work, sports activity, and smoking habits, were gathered from each individual. From these data the following variables were used for further analysis: I) Body mass index (BMI) = weight in kg/squared stature in m^2; II) Education (three categories): 1. Primary school (8 years education); 2. High school (12 years education); and 3. College and university education; III) Occupation (six categories): 1. Persons with a university diploma (e.g., physicians, engineers, economists); 2. Teachers; 3. Skilled workers and technicians; 4. Unskilled workers; 5. Farmers; and 6. Retired; IV) Physical exertion at work (four categories): 1. Hard physical exertion; 2. Moderate physical exertion; 3. Work standing up, but without effort; and 4. Sedentary work; V) Sports activity (two categories): 1. Not engaging in sports; and 2. Regularly engaging in sports; and VI) Smoking habits (three categories): 1. Nonsmokers; 2. Smokers (<19 cigarettes a day); and 3. Heavy smokers (20 or more cigarettes a day).

Statistical Analysis

At the beginning of the study, two-way ANOVA of plasma fibrinogen level, with sex as a first factor and each of the socio-demographic variables as a second factor, were performed. Not all the socio-demographic covariables were complete: education and physical exertion had missing values. The "missings" were included as an extra category and used in the model to achieve the equal number of subjects in each analysis. Linear correlation between fibrinogen level vs. age and BMI was also tested. Multiple regression analysis was then performed to examine the independent quantitative contribution of variables that showed a significant association with fibrinogen level in two-way ANOVA. Age and BMI were introduced in a regression analysis as quantitative variables; sex and all other categorical variables were entered as "dummy" variables, with

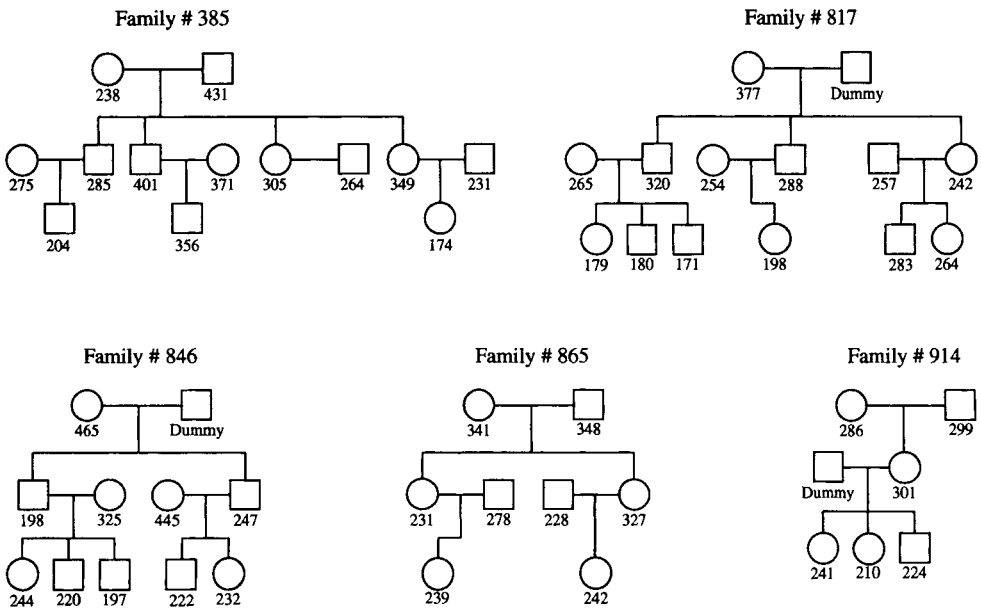

Fig. 1. Examples of complex pedigrees used in genetic analysis of fibrinogen level variation. Numbers below symbols are fibrinogen concentrations in mg/l.

the most numerous category used as a control group (SPSSx).

Fibrinogen values adjusted on the respective significant terms were subjected to further analysis. As shown in Figure 2, the distribution of adjusted fibrinogen values was significantly skewed. Therefore, the first step in genetic analysis was to determine whether the observed distributions represented a mixture (i.e., commingling) of 2 or 3 normal or skewed distributions, or a single skewed distribution [Lalouel, 1983]. According to the commingling model [Nirmala et al., 1992], the distribution of quantitative trait X is formed by the commingling of three conditional distributions, associated with the occurrence of a major gene locus with two alleles A and B. Allele A lowers, and B raises, the level of variable X. The three conditioned distributions for the hypothetical genotypes AA, AB, and BB have means m1, m2, and m3, respectively. Their frequency in the population is assumed to correspond to the Hardy-Weinberg equilibrium, i.e., q^2, $2q(1 - q)$, and $(1 - q)^2$, which corresponds to the areas under the three component distributions. Deviations from the expected genotype-specific means are assumed to be attributable to the additive effects of unlinked polygenic loci and random environmental effects.

The full model incorporates the following parameters: U, overall mean of the distribution of the variable X in the entire population, which is a mixture of three distributions corresponding to three genotypes, AA, AB, and BB; V, variance of the normally distributed variable X; D, dominance or recessiveness, i.e., relative position of the heterozygote AB; T, a measure of the displacement between the two hypothetical homozygous means AA vs. BB; Q, allele frequency; and P, Box-Cox power transformation of X [MacLean et al., 1976]. For further details, see Nirmala et al. [1992].

Genetic Analysis

The mixed model of inheritance [Morton and MacLean, 1974; Lalouel, 1983], as implemented in the modified version of the computer program MAN-2 [Ginsburg et al., 1986], was used in the present study. Briefly, the model assumes that the phenotype of the quantitative trait X results from the simultaneous contributions of a major locus, a multifactorial transmissible component, and the random environment. A major gene effect results from the segregation, at a single major locus with two alleles at frequency q and 1 − q, respectively. The model also assumes Mendelian transmission of alleles from generation to generation (denoted by tr1, tr2, and tr3, respectively), i.e., genotypes AA, AB, and BB transmit allele A with a probability of tr1 = 1, tr2 = 0.5, and tr3 = 0, as expected under Mendelian laws. In addition, the model incorporates parameters M_{AA}, M_{AB}, and M_{BB}, as well as V_{AA}, V_{AB}, and V_{AB}, estimating genotype-specific means and variances.

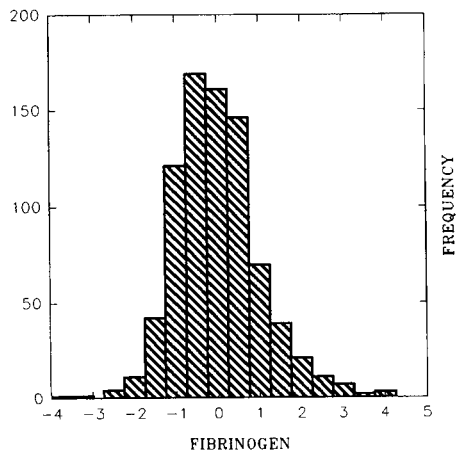

Fig. 2. Frequency distribution of Z-transformed plasma fibrinogen concentrations, adjusted for age and sex in total sample (N = 808). Descriptive statistics: mean = 0.000, SD = 0.999, mode = 1.071, median = −0.075, skewness ± SE = 0.658 ± 0.086, kurtosis ± SE = 1.273 ± 0.172.

Nonmajor gene multifactorial effects are due to effects of minor genes, common familial environment factors, and other unidentified environmental influences. The first two factors cause correlations between relatives. Three different correlations are introduced: 1) between spouses (r_{sps}); 2) parent-offspring ($r_{p/o}$); and 3) between sibs (r_{sib}). No supposition about their additive polygenic or other natures is made.

It is supposed that a correlation between any two members of a large pedigree can be nonzero, but any partial correlation between trait modifications in members of two different nuclear pedigrees, included in the larger one, equals zero. This means that no common genetic or environmental factors directly affecting these individuals are supposed. The trait modifications in them correlate indirectly, through individuals that are members of these two nuclear pedigrees simultaneously.

Let $X_m = (X_{1m}, X_{2m}, \ldots, X_{nm})$, $T_m = (t_{1m}, t_{2m}, \ldots, t_{nm})$, and $G_m = (g_{1m}, g_{2m}, \ldots, g_{nm})$ be sets of phenotypic values, ages, and genotypes in n members of an m-th component nuclear pedigree. It is supposed that joint distribution of modification $f(X_m/G_mT_m)$ is n-dimensional normal, with correlation matrix expressed through the three above-defined parameters r_{sps}, $r_{p/o}$, and r_{sib}.

This model of major gene control of a quantitative trait is determined by a set of 13 parameters. Some of the parameters may be set different for the two sexes. Partial variants of this model can be produced by special suppositions about parameter values.

As to transmissibility of fibrinogen, various hypotheses could be assessed in connection with the general model. Our Mendelian model assumes Mendelian transmission as described above, and therefore tr1, tr2, and tr3 were constrained to the expected values of 1, 0.5, and 0, respectively. All other parameters were estimated. In the present study we evaluated the following hypotheses, within the constraints of their respective parameters: 1) Mode of inheritance does not deviate from Mendelian transmission (general model). This hypothesis was tested by estimating all three transmission possibilities (i.e., tr1, tr2, and tr3) in addition to all other parameters mentioned above. 2) No genetic transmission is detectable (i.e., transmission probabilities are all the same (tr1 = tr2 = tr3) regardless of parental or offspring genotypes. 3) There are no within-genotype family correlation(s). In this model several submodels were tested, i.e., a) $r_{sps} = r_{p/o} = r_{sibs} = 0$; b) no

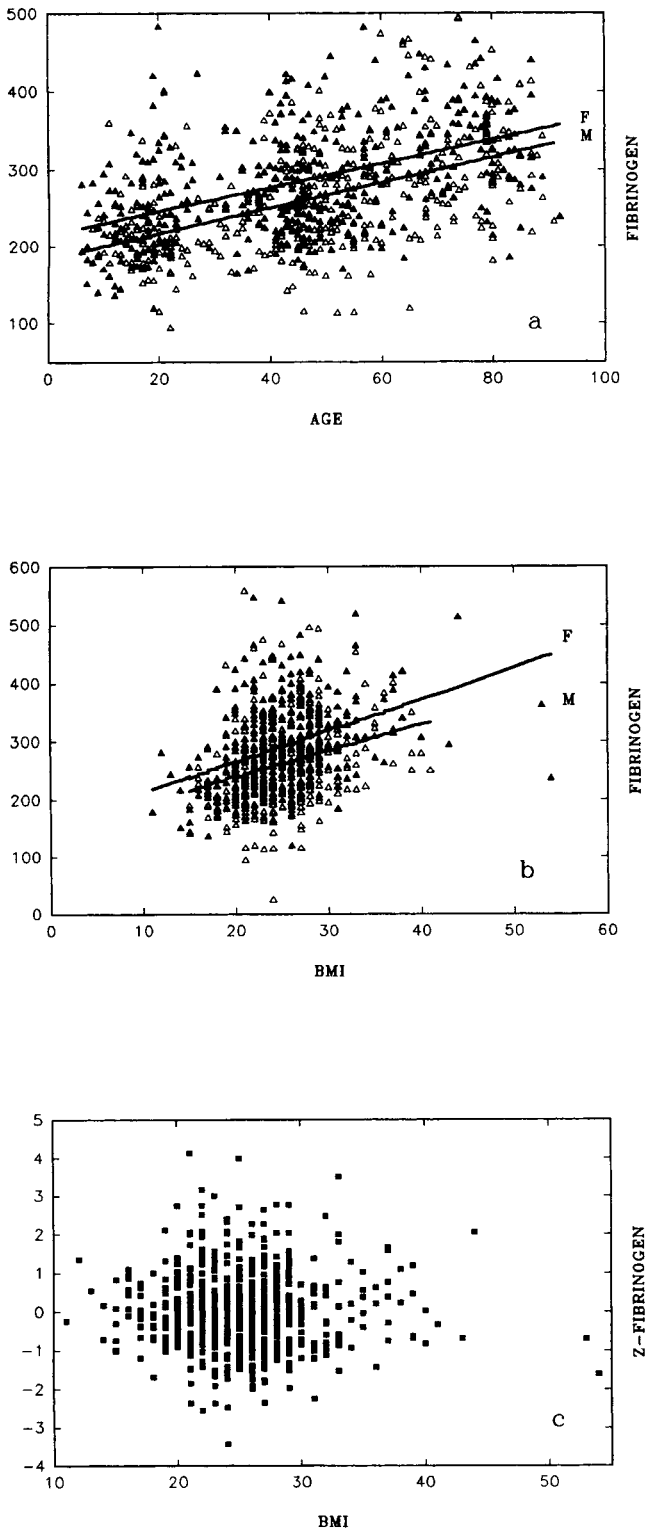

Fig. 3. **a:** Relationship between plasma fibrinogen level and age in total sample, according to sex. Regression equation for males: Fibrinogen = 184.1 + 1.653 age, $r = 0.501$, $P < 0.001$. Regression equation for females: Fibrinogen = 214.9 + 1.553 age, $r = 0.453$, $P < 0.001$. The t-test of the hypothesis that there is no linear relationship between fibrinogen and age was strongly rejected for both sexes (t_m = 11.4, t_f = 10.3, $P < 0.001$ for males and females respectively). Examination of the bivariate plot of the residuals against the predicted values did not detect any pattern. **b:** Relationship between plasma fibrinogen level and BMI in total sample, according to sex. Regression equation for males: Fibrinogen = 148.9 + 4.524 BMI, $r = 0.271$, $P < 0.001$. Regression equation for females: Fibrinogen = 157.4 + 5.374 BMI, $r = 0.387$, $P < 0.001$. The t-test of the hypothesis that there is no linear relationship rejected the hypothesis strongly (t_m = 5.2, t_f = 7.7, $P < 0.001$). Examination of the bivariate plot of the residuals against predicted values did not detect any pattern. **c:** Lack of relationship between BMI and plasma fibrinogen level residuals, adjusted for age and sex.

residual spouse correlation; c) no residual parent-offspring correlation; and d) no residual sibling correlation. In submodels b–d, the respective correlation was restricted to zero, while others were set free. 4) The allelic interactions within the major gene show complete dominance, i.e., $M_{AB} = M_{BB}$. 5) There is complete recessiveness, i.e., $M_{AB} = M_{BB}$. 6) There is codominance, i.e., $M_{AB} = 0.5 (M_{AA} + M_{BB})$. The Mendelian model, as well as hypotheses 2–6, are submodels of the general model and were compared to the latter, in order to choose the best and most parsimonious one. Analysis was performed in two stages: 1) We did not consider within-genotype family correlations. 2) Family correlations were included in the analysis (complex segregation analysis). The above-described general and partial models were constructed in each stage of analysis.

Hypothesis testing relied on the use of the likelihood ratio test to contrast pairs of models. Under the null hypothesis, the distribution of twice the difference in log likelihood of the model with the given number of parameters relative to the model with unrestricted parameters was approximated by chi-square distribution. Degrees of freedom (df) for this statistical test were equal to the difference in the number of restricted parameters between two hypotheses. The likelihood of any pedigree without loops was calculated through a successive convolution of nuclear pedigrees, ordered in such a way that at each stage of the convolution there was at least one nuclear pedigree connected to the large one by only one of its members. The major gene model was accepted if 1) the model variant without the major gene was rejected; 2) Mendelian transmission was not rejected; and 3) genetic prediction of individual trait values was more accurate than that performed by a multivariate regression technique [Ginsburg et al., 1986]. We also used Akaike information criteria (AIC) to choose the most parsimonious and best-fitting model [Akaike, 1974]. An AIC value was computed for each particular model as follows: 2(−loge likelihood + n), where n is the number of estimated parameters.

RESULTS

Confounding Variables

Simple univariate analysis of plasma fibrinogen level variation showed highly significant differences ($P < 0.001$) in mean values between men ($\bar{X}$ = 263.6, SD = 71.3, N = 396) and women ($\bar{X}$ = 285.9, SD = 74.9, N = 411). Fibrinogen levels increased considerably with age in both sexes. Pearson correlation was 0.501 ($P < 0.001$) and 0.453 ($P < 0.001$) for males and females, respectively, and the correlation was linear (Fig. 3a). To

illustrate this relationship, note that mean fibrinogen levels were 216.9 and 244.2 in the sample of young (<30 years old) men and women, vs. 270.3 and 301.1 in the older cohort.

Table I provides the results of two-way ANOVA of plasma fibrinogen level, with sex as first factor and the respective confounding variable as second factor. In all analyses, females had significantly higher fibrinogen values on average. All potential environmental variables also showed significant heterogeneity with respect to fibrinogen level. Note, for example, that heavy smokers (20 or more cigarettes a day) showed much higher mean values of fibrinogen, while individuals engaged in sports and/or having higher education possessed significantly lower levels than their respective groups for comparison. No significant two-way interaction terms were detected in any of the analyses reported.

We also examined the correlation between body weight, height, and BMI on the one hand, and fibrinogen level on the other. Height showed no correlation; body weight and BMI were significantly correlated to fibrinogen (0.13 and 0.27 in men, and 0.33 and 0.39 in women). Since correlation between weight and BMI was very high, we selected only BMI for further multivariate analysis. A plot showing the relationship between plasma fibrinogen levels and BMI is seen in Figure 3b. Again, the hypothesis of nonlinear relation was rejected.

Table II displays the outcome of a multiple linear regression model that included prespecified established life-style correlates of fibrinogen (Table I), age, sex, and BMI. Fibrinogen was higher in females than in males, and was associated positively and independently with age and BMI. These three variables collectively explained about 28.4% of the total variation in plasma fibrinogen level, with age alone contributing about 22%. All other variables, including smoking, had no significant independent effect on fibrinogen variation and therefore were not retained in the final regression equation.

The original fibrinogen values were adjusted for the significant terms and saved for further analyses. However, since 124 individuals were not measured for BMI in the present sample on the one hand, and the independent contribution of BMI to explanation of fibrinogen level, on the other hand, was 2.6% (Table II and Fig. 3c), the following genetic analysis was performed twice. In the first analysis (N = 684) fibrinogen levels were adjusted for age, sex, and BMI; in the second analysis (N = 757), they were adjusted only on age and sex.

Family Correlations

The parameter estimates obtained in genetic analysis performed on two samples were virtually the same. We therefore report here and in further analyses the results obtained on a larger sample (N = 757). Table III shows Pearson correlations between spouses, each parent, and mean offspring, and mean correlation for each possible pair of siblings, weighted on number of siblings per family, regardless of sex. All correlations, including marital, were positive and, excluding the former, were statistically significant (Table III). Note that father/offspring, mother/offspring, and sib correlations varied between 0.223–0.331. These differences, however, were statistically not significant ($\chi^2 = 0.97$, $df = 2$, $P > 0.25$).

TABLE I. Two-Way ANOVA of Plasma Fibrinogen Levels in Study Population*

	Men			Women			F-ratio	
	X	SD	N	X	SD	N	Sex	Environment factor
Smoking								
1	260.5	69.6	331	286.0	76.1	365	20.46, $P < 0.001$	5.81, $P = 0.003$
2	250.1	72.4	35	268.5	63.4	25		
3	303.0	87.8	31	302.3	64.0	21		
Sports								
1	273.0	74.6	272	293.1	73.7	256	22.81, $P < 0.001$	22.21, $P < 0.001$
2	240.9	61.6	125	273.8	75.5	155		
Occupation								
1	261.7	66.0	261	286.9	72.8	236	18.91, $P < 0.001$	40.64, $P < 0.001$
2	350.4	134.2	3	300.8	76.0	42		
3	215.5	38.2	72	233.0	53.7	73		
4	295.5	141.8	4	276.0	69.3	3		
5	321.3	78.9	57	338.4	63.8	57		
Exertion								
1	274.5	73.3	117	306.1	71.2	41	22.66, $P < 0.001$	3.36, $P = 0.010$
2	255.7	64.9	131	285.3	77.6	127		
3	283.2	84.1	35	293.0	76.5	79		
4	251.7	71.8	82	277.2	69.4	123		
Missing	256.6	78.0	32	278.5	80.3	11		
Education								
1	254.2	67.9	44	269.0	91.4	44	22.40, $P < 0.001$	4.31, $P = 0.005$
2	263.6	73.9	244	289.2	73.1	211		
3	255.3	61.0	88	284.9	72.4	145		
Missing	314.9	85.6	21	299.5	65.3	11		

*Labels for covariates are given in Materials and Methods.

TABLE II. Multiple Regression Analysis of Plasma Fibrinogen Level on Age, Sex, and Potential Environmental Factors in Study Population*

Variable	B	±	SE B	Corr	Part	Significance	dR^2
Age	1.45		0.12	0.47	0.42	$P < 0.001$	0.223
Sex	30.71		4.88	0.16	0.23	$P < 0.001$	0.035
BMI	2.66		0.53	0.31	0.19	$P < 0.001$	0.026
Constant	95.98		15.40			$P < 0.001$	0.284[a]

*B ± SE B is the regression coefficient and its standard error. Corr, initial Pearson correlation; Part, partial correlation; dR^2, change of multiple determination coefficient at each step of regression.
[a] Final value of multiple determination coefficient.

The midparent/midchild correlation of 0.382 was considerably lower than the h^2 value expected from sibling or parent/offspring correlations (0.570 and 0.554, respectively). This difference is in accord with the positive correlation between spouses, indicating the possible existence of a common family environment and/or some assortative mating or inbreeding. The regression of offspring on single parents and the full-sib correlation are both affected by assortative mating or inbreeding, i.e., by term $1 + r_{sps}$, while midparent/offspring correlation is not [Falconer, 1989]. Thus, the raw material correlations indicated the possible existence of a significant additive genetic heritability and/or major single locus effects, with no dominance and no transmissible common sibling environment. The data also showed absence of any strong maternal effects, while some assortative mating (or rather inbreeding) and/or common sibling environment. The data also showed absence of any strong maternal effects, while some assortative mating (or rather inbreeding) and/or common family environment was possible.

Commingling Analysis

As already mentioned, age- and sex-adjusted sample skewness of plasma fibrinogen level was statistically significant (Fig. 2). In order to test whether the distribution of this physiological trait represented a mixture (i.e., commingling) of 2 or 3 components, commingling analysis was applied [Lalouel, 1983]. Table IV shows that the hypothesis that the present sample was taken from a single normal distribution is strongly rejected ($P < 0.001$). The hypothesis that single skewed distribution fits the observed data well is also unacceptable ($P < 0.05$) on the basis of maximum log-likelihood ratio. Testing the hypotheses of the mixture of two normal or two skewed distributions showed that these models fit fibrinogen data, as well as did both three-distribution models, i.e., in all four tests the log-likelihood estimates of parameters were virtually the same. However, by AIC, the mixture of two normal distributions was the most parsimonious model for the observed distribution of fibrinogen. Thus, commingling analysis indicated the possible involvement of a major gene in determination of plasma fibrinogen variation, although the discrimination between various models was not clear-cut.

Segregation Analysis

Preliminary genetic analysis showed that the most parsimonious, best-fitting model for plasma fibrinogen level transmissibility is in accord with the Mendelian hypothesis, with nonequal genotype-specific variances. However, the variances clearly showed a positive relationship with respective genotype means. To avoid these possible allometric correlations, the data were log-transformed. Below, we provide results of analyses conducted on the log-transformed data. Table V gives parameter estimates and twice-log-likelihood values (lnLH) for various genetic models.

We started with classical segregation analysis (model 1) with transmission probabilities tr1, tr2, and tr3 restricted to 1, 0.5, and 0, respectively, and equal genotype variances. The log-likelihood value of this model was virtually the same as in the general model, where all three transmission probabilities were set free (model 2). The model rejecting Mendelian hypothesis was significantly worse ($P < 0.01$) than the general one (model 3). The Mendelian model was significantly improved ($\chi^2 = 25.3$; $df = 2$, $P < 0.001$), when estimates of intragenotype variances were freed (model 4), and the latter did not differ from the similar model except for transmissibility probabilities set free (model 5). Thus we can accept the Mendelian hypothesis for fibrinogen transmission, with genotype-specific variances.

The next stage of analysis involved complex segregation (Table V). Here we tested in a stepwise manner the importance of each of three possible residual family correlations, i.e., between spouses, between parent and offspring and between sibs. The first and third correlations were low, 0.0742 and 0.0168, respectively, and statistically not significant (models 6 and 8). Estimation of parent/offspring correlation (model 7) significantly improved the Mendelian model; assuming this correlation equal to zero, then $\chi^2 = 5.4$, $df = 1$, and $P = 0.02$. Further improvement of the model was achieved when residual parent-offspring and sib correlations were estimated simultaneously along with other parameters, except for transmission parameters which were restricted to Mendelian probabilities, and marital corre-

TABLE III. Family Correlations in Plasma Fibrinogen Level Adjusted for Age and Sex

	Father	Mother	Midparent	Offspring
Mother	0.122, NS[a]	1.000		
Midpar.	0.749**	0.745**	1.000	
Offspr.	0.331**	0.223*	0.382**	0.285*

* $P < 0.01$.
** $P < 0.001$.
[a] NS, no significance ($P > 0.05$).

TABLE IV. Commingling Analysis of Plasma Fibrinogen Level Distribution, Adjusted for Age and Sex

No.	Model	V	U	D	T	Q	P	F	AIC
1.	3 skewed	0.480	−0.044	0.147	2.456	0.290	0.770	617.26	629.3
2.	3 normal	0.498	−0.026	−0.005	2.370	0.292	[1.000]	617.49	627.5
3.	2 skewed	0.507	−0.042	[0.000]	2.286	0.293	0.802	617.25	627.3
4.	2 normal	0.498	−0.027	[0.000]	2.375	0.292	[1.000]	617.50	625.5[a]
5.	1 skewed	0.871	−0.162	[0.000]	[0.000]	[0.000]	−0.841	624.90*	630.9
6.	1 normal	0.938	−0.027	[0.000]	[0.000]	[0.000]	[0.000]	657.42**	661.4

* $P < 0.05$, significantly different from general model (3 skewed distributions).
** $P < 0.001$, significantly different from general model (3 skewed distributions).
[a] Indicates most parsimonious model by AIC. For definitions of parameters, see Materials and Methods.

lation, restricted to zero. The log-likelihood ratio estimate of this model (model 9) was significantly better than in models 7 and 8 ($\chi^2 = 4.0$ and 6.1, $df = 1, P < 0.05$). As seen in Table V, the obtained estimates of parameters in model 9 show that within the major gene, the allele responsible for high values of plasma fibrinogen level is codominant, its frequency in the Israeli population is about 67%, and the genotypes have different variances, with the largest variance for the homozygote AA. This model also retains two residual family correlations: the very small sibling correlation, and the considerably larger correlation between parent and offspring.

The comparison of this model with the one where transmissibility probabilities were set free (model 10) provided no evidence to reject the Mendelian hypothesis ($\chi^2 = 0.6$, $df = 3$, $P > 0.50$). In contrast, the environmental model (model 11) with $tr_1 = tr_2 = tr_3$ was strongly rejected ($\chi^2 = 18.6$, $df = 3, P < 0.001$). Finally, three more models were tested (not shown). They differed from the one (model 9) under the consideration only in terms of an allele interaction within the major locus. The first model assumed that allele B was recessive, the second, that the allele responsible for high values of fibrinogen level was dominant, and the third, that the alleles were codominant. The estimates of the third model were virtually the same as in model 9, and hence it was in full agreement with the hypothesis of codominant inheritance of fibrinogen concentrations. The lnLH in two other models was significantly higher, and they were both therefore rejected.

DISCUSSION

As mentioned in the Introduction, several recent prospective investigations have reported that higher plasma fibrinogen concentrations are associated with a greater risk of atherosclerotic disease. To discover the characteristics and, in particular, the genetic factors that may influence fibrinogen variation, the present three-generation offspring study was undertaken. Our study had the advantage that a large number of subjects with fibrinogen values could be used in the analysis, including some large families with quite complex pedigrees (Fig. 1). The families were collected in a nonselective way, and all individuals were healthy upon entrance examination. Additionally, information on sociodemographic variables and life-style factors were available and allowed us to investigate the influence of nongenetic components, and to adjust the analysis accordingly.

The results of our two-variate statistical analysis were in agreement with other studies [e.g., Lee et al., 1990; Folsom et al., 1991, 1993; Ernst, 1993] that showed significant association of fibrinogen level with age, sex, and BMI of an individual, as well as with several life-style characteristics such as sports activity, occupation, and smoking. However, in contrast with the above-mentioned studies, our multivariate consideration established that only the first three factors exerted significant and independent contributions to fibrinogen variation. The relationship between fibrinogen and other factors, including smoking, could be explained by age and BMI. Thus, for example, when we excluded BMI from the multiple regression analysis, sports activity was retained. However, <1.5% of the total variation was attributable to an independent effect of this factor.

Univariate linear correlation analysis showed that the relationship between fibrinogen level and age and BMI, respectively, did not significantly deviate from linearity assumption (Fig. 3a,b). However, the correlation between fibrinogen residuals (adjusted for age and sex) showed only very slight correlation with BMI (Fig. 3c). Since a considerable number of individuals were not measured for BMI, we decided to neglect this correlation in genetic analysis. We can note here that our estimates are very similar to those obtained by Hamsten et al. [1987]. The multiple determination coefficient for age and sex effects on plasma fibrinogen level in the entire sample in Hamsten et al. [1987] was 0.29, vs. 0.25 in our study. The contribution of BMI and smoking in Hamsten et al. [1987] comprised 0.032, vs. 0.026 in our study.

The family correlations in Hamsten et al. [1987] were also extremely reminiscent of what we obtained in the present analysis. The most parsimonious solution of the path analytic model in Hamsten et al. [1987] indicated that additive genetic heritability was substantial, about 0.50, while estimates of special maternal influences and marital correlation were virtually zero. Results of the present genetic analysis are also in very good agreement with the aforementioned family correlations. Thus, for example, the marital correlation detected in our correlation analysis (0.122) was not statistically significant. This latter finding was confirmed in segregation analysis. It is interesting to note also that the double parent-offspring correlation (h^2) was considerably higher than the midparent-midchild correlation, and that sibling correlation was not higher

TABLE V. Genetic Analysis of Logarithm-Transformed Plasma Fibrinogen Level in Israeli Pedigrees*

Genetic parameters	1	2	3	4	5	6	7	8	9	10	11
Q	.4065	.4047	.2218	.7781	.7832	.7381	.7646	.7808	.6670	.6665	.3763
Mean											
AA	−.6245	−.6240	.0949	−1.1511	−1.5120	−.5262	−1.1377	−1.5292	−1.3990	−1.3770	.1235
AB	.0577	.05954	−.4421	−.5240	−.5192	−.4957	−.4398	−.5425	−.4576	−.4385	−.2393
BB	1.1115	1.1116	1.9500	.4103	.4062	.4050	.3883	.4096	.3901	.3872	.3892
Variance											
AA	.6533[a]	.6536[a]	.7871[a]	1.3360	1.3333	1.3048	1.4141	1.3380	1.4083	1.4152	1.8233
AB	.6533[a]	.6536[a]	.7871[a]	.3259	.3254	.3750	.3367	.3130	.3245	.3364	.4445
BB	.6533[a]	.6536[a]	.7871[a]	.8460	.8519	.7841	.9084	.8413	.9013	.9102	.8178
Family correlations											
r_{sps}	[.0000]	[.0000]	[.0000]	[.0000]	[.0000]	.0742	[.0000]	[.0000]	[.0000]	[.0000]	[.0000]
r_{po}	[.0000]	[.0000]	[.0000]	[.0000]	[.0000]	[.0000]	.2663	[.0000]	.2586	.2606	.4079
r_{sib}	[.0000]	[.0000]	[.0000]	[.0000]	[.0000]	[.0000]	[.0000]	.0168	.0214	.0212	[.0000]
tr_1	[1.0000]	1.0000	.8317[a]	[1.0000]	1.0000	[1.0000]	[1.0000]	[1.0000]	[1.0000]	1.0000	.4850[a]
tr_2	[.5000]	.4961	.8317[a]	[.5000]	.5234	[.5000]	[.5000]	[.5000]	[.5000]	.5084	.4850[a]
tr_3	[.0000]	.0000	.8317[a]	[.0000]	.0000	[.0000]	[.0000]	[.0000]	[.0000]	.0000	.4850[a]
−2ln − LH	1830.7	1830.4	1846.7	1805.4	1805.3	1803.0	1800.0	1802.1	1796.0	1796.6	1815.2

* For parameter definitions, see Materials and Methods. Square brackets indicate parameters set to values in the model.
[a] Parameters constrained to equal values.

than parent-offspring correlation. Our best-fitting model shows that, apart from major gene effect, there was a considerable residual parent-offspring correlation (0.26), while the residual sib correlation was virtually zero. This means that the existence of a second gene involved in determination of plasma fibrinogen variation is rather unlikely. However, existence of some kind of common family environment is possible. Moreover, testing the hypotheses with equal and unequal within-genotype variances allows us to accept Mendelian transmission parameters with unequal genotype-specific variances. The fact that the second model is better may be indicative of a different genotype × environment (or age) interaction. This model has not been tested in the present analysis.

In general, previous research has documented great heterogeneity in results of genetic analysis of plasma fibrinogen concentration. Although several studies have clearly indicated the familial aggregation of fibrinogen levels [Hamsten et al., 1987; Humphries et al., 1987; Mihai and Jansco, 1991; Dubrey et al., 1994], others have concluded that there is no detectable genetic influence on fibrinogen variation [Thomas et al., 1991; Connor et al., 1992; Reed et al., 1994]. Friedlander et al. [1995] recently performed complex segregation analysis in a sample of 82 Israeli kindreds. They found strong evidence of significant genetic effect. However, no discrimination between models assuming major gene effect vs. polygenes only was possible in this study. The present study indicates the existence of a major gene effect with a codominant allele determining high levels of plasma fibrinogen. In the Israeli population the frequency of this allele approaches 67%. The proportion of the variance of fibrinogen concentration that could be explained by a major locus comprises about 39%.

This latter finding supports the estimate by Hamsten et al. [1987] of genetic effects on fibrinogen variation, but contradicts that reported by Humphries et al. [1987]. The latter study showed an association between certain RFLP polymorphisms (by β-fibrinogen probe) and plasma fibrinogen level. However, genetic differences account for only 15% of the total phenotypic variance. Notable for showing a small genetic component was the European Atherosclerosis Research Study [Humphries et al., 1995], in which genetic effects on fibrinogen level in the offspring of fathers with myocardial infarction, as well as control subjects from five different regions of Europe, were investigated. Again, the β-fibrinogen gene polymorphism was studied, and in agreement with Humphries et al. [1987] and our study, one of the homozygotes in the control group showed a clearly elevated fibrinogen level. Using the data on control subjects in Humphries et al.[1995] (Table II), we computed the relative magnitudes of the variance components between these homozygotes and opposite homozygotes, and heterozygotes. The proportion of variation among the groups (genotypes) comprised only about 3%.

However, the situation was different in individuals with a family history of ischemic heart disease (IHD), and in women using oral contraceptives. The association between genotype and fibrinogen level disap-

peared in some cases, or changed the sign in others. These findings led us to the assumption that the DNA polymorphisms studied up to now have had little effect on the fibrinogen level, and at least one additional unknown locus, probably with regulatory functions, should exist.

ACKNOWLEDGMENTS

This research was supported by a grant from VerUm, Stiftung fur Verhalten and Umvelt, München, Germany, and was partly supported by Israeli Ministry of Science and Arts grant 6337-1-9. We thank Dr. E. Ginzburg and two anonymous reviewers for constructive and useful comments and suggestions. We are grateful to Mrs. G. Hay and Mrs. M. Goldschmidt-Nathan for typing and technical assistance during the preparation of this manuscript.

REFERENCES

Akaike H (1974): A new look at the statistical model identification. IEEE Trans Automat Control AC 19:716–723.

Berg K, Kierulf P (1989): DNA polymorphisms at fibrinogen loci and plasma fibrinogen concentration. Clin Genet 36:229–235.

Brunner D, Weisbort J, Meshulan N, Schwartz S, Gross J, Saltz-Rennert H, Altman S, Loebl K (1987): Relation of total cholesterol and light-density lipoprotein cholesterol percentage to the incidence of definite coronary events. Twenty-year follow-up of the Conolo-Tel-Aviv prospective coronary artery disease study. Am J Cardiol 59:1271–1276.

Connor JM, Fowkes FGR, Wood J, Smith FB, Donnan PT, Lowe GDO (1992): Genetic variation at fibrinogen loci and plasma fibrinogen levels. J Med Genet 29:480–482.

Dang CV, Bell WR (1989): The normal and morbid biology of fibrinogen. Am J Med 87:567–576.

Dubrey SM, Reaveley DR, Seed M, Lane DA, Ireland H, O'Donnell M, O'Connor B, Noble MI, Leslie RDG (1994): Risk factors for cardiovascular disease in IDDM: A study of identical twins. Diabetes 43:831–835.

Ernst E (1990): Plasma fibrinogen—An independent cardiovascular risk factor. J Int Med 227:365–372.

Ernst E (1993): The role of fibrinogen as a cardiovascular risk factor. Atherosclerosis 100:1–12.

Falconer DS (1989): Introduction to Quantitative Genetics." London: Longman, pp 176–179.

Folsom AR, Wu KK, Davis CE, Conlan MG, Sorlie PD, Szklo M (1991): Population correlates of plasma fibrinogen and factor VII, putative cardiovascular risk factors. Atherosclerosis 91:191–205.

Folsom AR, Qamhieh HT, Flack JM, Hilner JE, Liu K, Howard BV, Tracy RP (1993): Plasma fibrinogen: Levels and correlates in young adults. Am J Epidemiol 138:1023–1036.

Fowkes FGR, Connor JM, Smith FB, Wood J, Donnan PT, Lowe GDO (1992): Fibrinogen genotype and risk of peripheral atherosclerosis. Lancet 339:693–696.

Friedlander Y, Elkana Y, Sinnreich R, Kark JD (1995): Genetic and environmental sources of fibrinogen variability in Israeli families: The Kibbutzim family study. Am J Hum Genet 56:1194–1206.

Ginsburg EKH, Fedotov AM, Chepkasov IL (1986): "Program Package for Testing Major Gene Models of Quantitative Trait, MAN-1." Novosibirsk: Institute of Cytology and Genetics Press, Russian Academy of Sciences, 1–23.

Hamsten A, DeFaire U, Iselius L, Blomback M (1987): Genetic and cultural inheritance of plasma fibrinogen concentration. Lancet 2(8566):988–991.

Hemker HC, Kop J, Willema GM (1979): Kinetic aspects of the interaction of blood clotting enzymes. The relation between clotting time and clotting velocity. Thromb Haemost 41:309–313.

Humphries SE, Dubowitz M, Cook M, Stirling Y, Meade TW (1987): Role of genetic variation at the fibrinogen locus in determination of plasma fibrinogen concentrations. Lancet 1(8548):1452–1455.

Humphries SE, Ye S, Talmud P, Bara L, Whilemsen L, Tiret L (1995): European atherosclerosis research study: Genotype at the fibrinogen locus (G-455-A B-Gene) is associated with differences in plasma fibrinogen levels in young men and women from different regions in Europe. Arteriosclerosis Thromb Vascular Biol 15: 96–104.

Kannel WB, Wolf PA, Castelli WP, Diagostino RB (1987): Fibrinogen and risk of cardiovascular disease. JAMA 258:1183–1186.

Lalouel JM (1983): Segregation analysis of familial data: the mixed model. In Morton NE, Rao DC, Lalouel JM (eds): "Methods in Genetic Epidemiology." Basel: Karger, pp 75–79.

Lee AJ, Smith WCS, Lowe GDO, Tunstall-Pedoe H (1990): Plasma fibrinogen and coronary risk factors: The Scottish heart health study. J Clin Epidemiol 43:913–919.

MacLean CJ, Morton NE, Elston RC, Yee S (1976): Skewness in commingled distributions. Biometrics 32:695–699.

Mihai K, Jancso T (1991): Plasma fibrinogen level in offspring of parents suffering from premature coronary artery disease. Orv Hetil 132:1861–1863.

Morton NE, Maclean CJ (1974): Analysis of family resemblance III. Complex segregation of quantitative traits. Am J Hum Genet 26: 489–503.

Nirmala A, Rice T, Reddy PC, Krishna KS, Ramana PV, Rao DC (1992): Commingling and segregation analysis of blood pressure in consanguineous and nonconsanguineous families from Andhra Pradesh, India. Am J Hum Biol 4:7093–716.

Reed T, Tracy RP, Fabsitz RR (1994): Minimal genetic influences on plasma fibrinogen level in adult males in the NHLBI twin study. Clin Genet 45:71–77.

Thomas AE, Greer FR, Kelleher CH, Wilkes HC, Brennan PJ, Meade TW, Humphries SE (1991): Variation in the promoter region of the beta fibrinogen gene is associated with plasma fibrinogen levels in smokers and non-smokers. Thromb Haemost 65:487–490.

Wilhelmsen L, Svardsudd K, Korsan-Bengtsen K, Larsson B, Welin L, Tibblin G (1984): Fibrinogen as a risk factor for stroke and myocardial infarction. N Engl J Med 311:501–505.